

Serial No.: 09/710,444

all right
acid sequence of the junction between Barnase and p3 sequences is shown in expanded view
(SEQ ID NO 77 and SEQ ID NO: 78, respectively)

REMARKS

The amendments directed herein are made in order to add SEQ ID NOs corresponding to the SEQ ID NOs in the accompanying Sequence Listing. The amendments add no new matter.

7/25/02
Date

Respectfully submitted:

Mark J. FitzGerald
Reg. No. 45,928 for
Kathleen M. Williams

Kathleen M. Williams
Kathleen M. Williams
Reg. No. 34, 380
Attorney for Applicant
Palmer & Dodge LLP
111 Huntington Avenue
Boston, MA 02199-7613
Tel: (617) 239-0451
Fax: (617) 227-4420



Series No.: 09/710,444

Version of amendments marked to show changes:

- Replace the paragraph at lines 14 to 23 on page 15 with the following replacement paragraph:

-- A sequence (PAGLSEGSTIEGRGAHE; SEQ ID NO: 1) comprising several proteolytic sites is inserted in the flexible glycine-rich region between the D2 and D3 domains of the phage p3. Incubation of the phage (fd-K108) under native conditions with trypsin, thermolysin or subtilisin now resulted in almost complete loss of infectivity (from 10^7 to < 10 TU/ml) and incubation with Glu-C and chymotrypsin resulted in a major loss (from 10^7 to 10^4 TU/ml). This indicates that these proteases cleave the new linker. However incubation with Factor Xa, Arg-C or thrombin did not lead to a loss in infectivity, despite the presence of potential cleavage sites for these enzymes. Presumably the presence of the D2 and D3 domains may block access or cleavage for these enzymes in the case of the present polypeptide.--

- Replace Table 4, on page 24, with the following replacement Table 4:

--Table 4

Table 4. Primer sequences

pklinker	5'GGCACCTCAGAACGGTACCCACCTCAGAGGCCGGCTGGG CCGCCACCTCAGAG 3' (SEQ ID NO: 2)
polyXafor	5'GGTGGCGGCCAGCCGGCCTTTCTGAGGGGTCGACTATAGAA GGACGAGGGGCCAGCGAAGGAGGTGGGGTACCCCTTCTGAGG GTGG 3' (SEQ ID NO: 3)
polyXaback	5'CCACCTCAGAAGGGGGTACCCACCTCCTTCGCTGGGCCCT CGTCCTTCTATAGTCGACCCCTCAGAAAGGCCGGCTGGGCCG CACC 3' (SEQ ID NO: 4)
fdPCRBack	5'GCGATGGTTGTTGTCATTGTCGGC 3' (SEQ ID NO: 5)
LIBSEQfor	5'AAAAGAAACGCAAAGACACCACGG 3' (SEQ ID NO: 6)
LIBSEQback	5'CCTCCTGAGTACGGTGATACACC 3' (SEQ ID NO: 7)
LSPAf or	5'GTAAATTCAGAGACTGCGCTTTCC 3' (SEQ ID NO: 8)
LSPAback	5'ATTTTCGGTCATAGCCCCCTTATTAG 3' (SEQ ID NO: 9)
Flagprimer	5'CAACGGGCGGCCGCGAGACTACAAGGATGACGACGACAAGG AAACTGTTGAAAGTTGTTTAGCAA 3' (SEQ ID NO: 10)
RECGLYfor	5'CCCCTCAGAAAGGCCGGCTGGGCCGCCGCCAGCATTGACAG GAGGTTTCAGG 3' (SEQ ID NO: 11)
RECGLYback	5'GAAGGAGGTGGGGTACCCGGTTCCGAGGGTGGTTCCGGTTC CGGTGATTTTG 3' (SEQ ID NO: 12)
delcKpn	5'CCCTCGGAACCGGTACCCAGCTGCTTCGTGGGCCC 3' (SEQ ID NO: 13)
Barnasefor	5'CTGGCGGCGGCCAGCCGGCCCTGCACAGGTTATCAACACG TTTGAC 3' (SEQ ID NO: 14)
BarnaseH102Aback	5'CTCGGAACCGGTACCTCTGATTTTGTAAAGGTCTGATAAGC G 3' (SEQ ID NO: 15)
villinfor	5'GGCGGCCAGCCGGCCTTTCTCTCTCTGACGAGGACTTCAAG GC 3' (SEQ ID NO: 16)
villinback	5'CCTCGGAACCGGTACCGAAGAGTCCTTTCTCCTTCTTGAGG 3' (SEQ ID NO: 17) --

-Replace the paragraph at page 30, lines 4-14 with the following replacement paragraph:

--1: TACGCCAAGCTTGCATGC (SEQ ID NO: 18);
2: CTGCACCTGGGCCATGG (SEQ ID NO: 19);
3: GATTACGCCAAGCTTTG (SEQ ID NO: 20);
4: GATTACGCCAAGCTTGCATGCANNDCTNTDTCAAGGAGACAGTCATAATGARRN
NBCTATTGSYAAYRSYASYAGBNTTGTTATTACTCSYANYCVNNCYGDCCATGG
CCCAGGTGCAGCTG (SEQ ID NO: 21);
5: GATTACGCCAAGCTTTGNNNNCTTTTTTWWGGAGATTTCACRTGARAARATTAT
TATTCSYAATTSYTTTAGTTSYTSYTTTCTWTGYGGYCCAGCCGGCCATGGCCCAGGT
GCA. (SEQ ID NO: 22)
6: CTTTATGCTTCCGGCTCG. (SEQ ID NO: 23)
7: CGGCCCCATTCAGATCC. (SEQ ID NO: 24)--

- Replace Table 7, on pages 35 and 36, with the following replacement Table 7. Because portions of the original text are underlined, the present amendments are indicated by double underlining.

112 GT TAGC CG CT GG CT GC CCC C A (SEQ ID NO: 34)

pelB MKYLLPTAAAGLLLLAAQPAMA (SEQ ID NO: 35)

17 KT AMVLVG PPGPS (SEQ ID NO: 36)

110 RG AMLVAG PIAPA (SEQ ID NO: 37)

112 RR VIAAVG LAPPT (SEQ ID NO: 38)

III-B. From library II

g3leader GAGC TT G A A (SEQ ID NO: 39)

5' AAGTTGNNNNCTTTTTTWWGGAGATTTCAACRTGARAARATTATTAT
(SEQ ID NO: 40)

19 GGGC TA A G G (SEQ ID NO: 41)

GC CC GT CC A C C (SEQ ID NO: 42)

TCSYAATTSYTTTAGTTSYTSYTTTCTWTGYGGYCCAGCCGGCCATGG CC3'
(SEQ ID NO: 43)

19 CT CC GT GC A T T (SEQ ID NO: 44)

g3 leader MKKLLFAIPLVVPF YAAQPAMA (SEQ ID NO: 45)

19 RR L P VA YVV (SEQ ID NO: 46)

--Replace the paragraph at lines 8-17 on page 38 with the following replacement paragraph. Please note that the sequences were presented in brackets in the original filed application. The brackets are replaced by parenthesis herein.

--The phage displaying the Stoffel fragment are incubated with primer **13** (TTT CGC AAG ATG TGG CGT) (SEQ ID NO: 47) comprising a 5' maleimidyl group and a 3' biotinylated nucleotide. After incubation the phage are captured on streptavidin-coated beads, with a yield of about 1-5% of infectious phage. This shows that primer can be chemically cross-linked to the phage, presumably via p8 protein as shown for the N-biotinoyl-N'(6-maleimidohexanoyl) hydrazide. The phage are then incubated with primer **1b** (GCGAAGATGTGG) (SEQ ID NO: 48) comprising a 5' maleimidyl group in the presence of biotin-dUTP **2** and template **3** (AAA TAC AAC AAT AAA ACG CCA CAT CTT GCG) (SEQ ID NO: 49). Capture of the phage is dependent on presence of **1b**, **2** and **3** (Table 8), but also on the inclusion of trypsin, which cleaves the helper phage to reduce non-specific phage isolation.--

- Replace the paragraph at page 39, lines 19-27 with the following replacement paragraph:

--For the cloning of (poly)-peptide encoding DNA fragments and their display for selection between barnase and p3, the phage fd-3 is constructed (Fig. 5). Phage fd-3 comprises the H1021A mutant of barnase N-terminally fused to the p3 gene of phage fd.TET. Between the codon for the last residue of barnase and the first residue of p3 is the nucleotide sequence *CTG GAG GCG GTG CGG CCG CA* (SEQ ID NO: 50). This sequence contains a PstI DNA restriction site (in italics) for insertion of DNA fragments flanked by PstI restriction sites. The sequence further introduces a frame shift between barnase and p3, which prevents expression of the correct p3 reading frame in fd-3. Phage particles of phage fd-3 therefore do not display the infection protein p3 and are non-infectious.--

- Replace the paragraph at page 40, lines 8-23 with the following replacement paragraph:

--Genomic DNA from the *E. coli* strain TG1 is amplified in 30 cycles of a polymerase chain reaction (PCR) with an annealing temperature of 48°C using the oligonucleotide SN6MIX (5'-GAG CCT GCA **GAG CTC** AGG NNN NNN-3'; SEQ ID NO: 51), which comprises 6 degenerate positions at the extendible 3' end to ensure random priming. In a second step of 30 PCR cycles with an annealing temperature of 52°C primary PCR products are extended by reamplification with the oligonucleotide XTND (5'-CGT GCG AGC **CTG CAG AGC TCA** GG-3'; SEQ ID NO: 52). Products with a length of around 150 bp from this reaction are purified from an agarose gel and reamplified in 30 PCR cycles using an annealing temperature of 52°C and the oligonucleotide XTND. These reamplified 150 bp fragments are partially digested with SacI (site indicated in bold in the oligonucleotides) and ligated for dimerisation. Ligated products are reamplified in a further 10 PCR cycles with an annealing temperature of 44°C followed by a 30 PCR cycles with an annealing temperature of 55°C using the oligonucleotide XTND. The annealing temperatures are chosen to discriminate against priming of the oligonucleotide in the middle of the dimerised fragments. The reaction product is size purified twice on an agarose gel to remove monomers and oligomers (non-dimers).--

- Replace the table on page 44 (Table 9) with the following replacement table:

--Phage clone	Proteolytic selection	Barstarbindg		Amino acid sequence of inserts
		-DTT	+DTT	
TA-1.2	1xTr	yes	no	LQSSGDCVIS DTCLAGMAEA AACEEKFSSQ NVGLTITVTP CLSSA (<u>SEQ ID NO: 53</u>)
TA-2.25	2xTr	yes	no	LQSSGCGSSG SSINCLPCGA TSRGTSPLAS GLPSSATIHC LSSA (<u>SEQ ID NO: 54</u>)
TA-2.26	2xTr	yes	no	LQSSGDSAGC KNMTGGRLYA HTLEAIIPGF AVSAPACEPA (<u>SEQ ID NO: 55</u>)
TA-2.27	2xTr	yes	yes	LQSSGCVRLK RTSVNHQPDA WPEPHLKAAC EPA (<u>SEQ ID NO: 56</u>)
TA-2.30	2xTr	yes	no	LQSSGCGSSG SSINCLPCGA TSRGTSPLAS GLPSSATVQC LSSA (<u>SEQ ID NO: 57</u>)
TB-1.10	1xTh	yes	yes	LQSSGKIVQA GANIQDGCIM HGYCDTDTIV GENGHIGLSS A (<u>SEQ ID NO: 58</u>)
TB-1.11	1xTh	yes	yes	no insert, Barnase & p3 in frame
TB-2.33	2xTh	yes	no	LQSSGVCVIS DTCLAGTAEA AACEEKFSSQ NVGHTITETP CLSSA (<u>SEQ ID NO: 59</u>)
TB-2.34	2xTh	yes	no	LQSSGCGSSG SSINCLPCGA TSRGTSPLAS GLPSSATIQ C LSSA (<u>SEQ ID NO: 60</u>)
TE-2.35	2xTh	yes	no	LQSSGQDSQR EHASHTAEDD CEDQTRIHQH IREVDFVDTP QEVDDCRAAL SSA (<u>SEQ ID</u> <u>NO: 61</u>)
TB-2.37	2xTh	yes	no	LQSSGCVRLK RTSVNHQPDA WPEPHLKAAC EPA (<u>SEQ ID NO: 62</u>)
TB-2.38	2xTh	yes	yes	LQSSGVRPA (<u>SEQ ID NO: 63</u>)
TB-2.39	2xTh	yes	no	LQSSGCGSS GSSINCLPCGA TSRGTSPLAS GLPSSATIQ CLSSA (<u>SEQ ID NO: 64</u>) --

- Replace the table at lines 12-29 on page 46 with the following replacement table:

Phage clone	Proteolytic selection	Barstarbindg +DTT	Amino acid sequence of inserts
B2-13 (SEQ ID NO: 65)	2xTr/Th	yes	LQSSGTEVDR GNQQHDTNDR DFTHTPLSS A
B2-14	2xTr/Th	yes	LQSSG5VAQG SSASVDVTAT NAVLSADSL SLGGGEPA (SEQ ID NO: 66)
B2-22	2xTr/Th	yes	LQSSGGAVAV TPGPVLSSA (SEQ ID NO: 67)
B2-23	2xTr/Th	yes	LQSSGHCRGK PVLCTHTA (SEQ ID NO: 68)
B2-15	2xTr/Th	yes	LQSSGVRPA (SEQ ID NO: 69)
B2-17	2xTr/Th	yes	no insert, Barnase & p3 in frame
B2-20,21	2xTr/Th	yes	no insert, Barnase & p3 in frame
B2-16,24	2xTr/Th	yes	LQSSGEPAPA HEAKPTEAPV AKAEAKPETP AHLSSA (SEQ ID NO: 70)
B2-18	2xTr/Th	no	LQSSGCVRLK RTSVNHQPDA WPEPHLKAAC EPA (SEQ ID NO: 71)
B2-19	2xTr/Th	no	LQSSGVVDWA KMREIADSIG AYLFVDMAHV AALSSA (SEQ ID NO: 72) --

- Replace the paragraph at page 47, lines 8-10 with the following replacement paragraph:

-- **Figure 2. The phagemid vectors pK1 and pK2.** These vectors contain a protease cleavable sequence between D2 and D3 of the phage p3 protein. In pK1, D2 + D3 are in frame; in pK2, D3 is out of frame. Nucleotide and amino acid sequence for the polylinker regions are shown for pK1 (SEQ ID NO: 73 and SEQ ID NO: 74, respectively) and pK2 (SEQ ID NO: 75 and SEQ ID NO: 76, respectively). --

- Replace the paragraph at page 47, lines 21-23 with the following replacement paragraph. Please note that number "43" was presented in brackets in the originally filed application. The brackets are replaced with parentheses herein.

-- Figure 5. The fd vector fd-3. The gene for the H102A mutant of Barnase is introduced by subcloning into fd-DOG (43) after PCR amplification with suitable oligonucleotides using the

Serial No.: 09/710,444

restriction sites ApaLI (at the Barnase 5' end) and NotI to create fd-3. The nucleotide and amino acid sequence of the junction between Barnase and p3 sequences is shown in expanded view (SEQ ID NO 77 and SEQ ID NO: 78, respectively).--